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14. ABSTRACT In this proposal, we will identify and clone a protein that can be used to generate infection-specific gene therapy vector. We expect that using this protein to modify various gene therapy vectors, we can specifically deliver cytotoxic genes into prostate cancer cells using systemic treatment, and eventually eradicate metastatic prostate cancer cells in patients. In summery, although we worked very hard to perform the studies we proposed, we failed to achieve the expected results. In our application, we actually know the risk of this project. However, if we could identify the protein that is responsible for tissue-specific gene delivery, we should be able to develop a prostate-specific. By comparing the risk and the potential achievement, you proposed this project. Now the results demonstrated that the project is more difficult than we expected. Although we did not achieve the expected results, our effort produced publishable results as shown by one published paper and one submitted manuscript.				
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Title: Identification of a Protein for Prostate-Specific Infection

PI: Shen Pang

Start date: Dec. 1, 2003

Grant number: W81XWH-04-1-0084

### **Introduction:**

Using gene therapy vectors to eradicate prostate cells is extensively studied. It is expected that if we can use a target-specific gene therapy to deliver cytotoxic genes into metastatic prostate cancer cells in patients, we can eradicate these malignant cells and cure the patients. To find an approach to generate infection-specific vectors is critical for target-specific cancer cell eradication. Because viral vectors infect cells by the binding of their surface proteins to their receptors on the target cells, it is expected that infection-specific vectors can be generated by modifying their surface proteins with proteins that can specifically bind to target cells. In our Preliminary Studies, we found that the lentiviral vector (an HIV-based retroviral vector) generated from a cell line derived from human oral tissue (HOT) can specifically infect LNCaP prostate cancer cells. Virus generated from other cell lines has much lower infectivity to LNCaP cells (200 to 1200-fold lower). These results strongly suggest that the HOT cells express a very specific membrane protein that can be picked by lentiviral vectors to modify their envelope. With such protein on its envelope, the viral vectors are able to specifically infect LNCaP cells.

If this is the case, we expect that a protein for generating infection-specific vector can be obtained by screen the cDNA library of the HOT cell line.

### **Body:**

The approved SOW is listed below.

Task 1. To prepare cDNA library in eukaryotic gene expression vectors (months 1-8).

Task 2. To perform first-round screening to identify the cDNA clone groups that contain the cDNA clones encoding the protein responsible for tissue-specific infection. (months 9-20)

Task 3. To perform the second round of screening to identify the individual clones that contain the cDNA encoding the protein responsible for tissue-specific infection (months 21-24)

Task 4. To sequence the identified gene (months 25-26)

Task 5. To use sequence analysis to characterize the identified gene (months 27-28)

Task 6. To generate deletions of the identified gene and to use them to confirm the functional domains of the identified gene (months 31-36)

To follow the schedule, we should complete Tasks 1-4. We actually achieved our goal with some modifications as described in the following sections.

### **Progress of our research:**

#### **For Tasks 1 and 2:**

Our plan was to screen 6,000 to 12,000 cDNA clones from the HOT cDNA library. During the first year, we have made a cDNA library. To check the quality of the cDNA library, we picked up clones to quantify the percentage of clones that contain cDNA inserts and the sizes of the cDNA inserts. We found that approximately 70% of our clones containing cDNA inserts. Most of the inserts are between 1.0 to 2.0 kb (Fig. 1). These sizes and percentage of our cDNA library fulfill our expectation. The total colonies in our cDNA library were approximately  $1 \times 10^5$ . We expected that the amount of the expected mRNA expressed in the HOT cell line was between 0.01 to 0.1% of the total mRNA. The minimal requirement of cDNA clone number is 1,000 to 10,000. Therefore, we believe the cDNA library that we generated is qualified for gene screening.

We have screened approximately 9,200 colonies by the end of the second funding year (Task 2).

However, we have not identified the cDNA clones that can specifically increase the transduction efficiency by lentiviral vectors to LNCaP prostate cancer cells. Therefore, although we followed the schedule to screen high numbers of cDNA clones, we could not just go to the study of Task 3.

### For Tasks 3 to 6

There are two explanations why we failed to identify the gene responsible for tissue specific transduction of prostate cancer cells: 1) Our cDNA library may not cover all the genes that are expressed in HOT cell line, and 2) the ligand of the protein that is responsible for lentiviral vector from HOT cells may contain two or even more subunits. When we use cDNA clones to prepare lentiviral vectors for gene delivery, the probability to have both cDNAs in the same viral proportion is very low. Therefore, we made another cDNA library and screened another 4,000 cDNA colonies. However, with such additional screening, we still have not

found any cDNA clones can significantly increase viral vector gene delivery into LNCaP cells. We hypothesized that the expected ligand protein that is responsible for efficacious infection of LNCaP by the HOT-cell-derived lentiviral vector may have two or even more subunits. Current screening strategy would not be able to identify such genes because the probability to have both subunits in one vector preparation was very low. The vectors we generated may have either subunit A or B, but not both. In such case, the prepared vectors could not demonstrate significant increase in delivering genes into prostate cells.

In such context, we modified our strategy to develop prostate-specific gene delivery vectors. One approach is to use peptides derived from

prostate-specific membrane protein to increase lentiviral vector gene delivery. We have found that LNCaP and other prostate cancer cells express high levels of claudin-7 membrane protein. Claudin-7 is a membrane protein related to PSA expression in prostate cells (Zheng JY, Yu D, Foroohar M, Ko E, Chan J, Kim N, Chiu R, Pang S. Regulation of the Expression of the Prostate-specific Antigen by Claudin-7. **J. Membrane Biol.** 2003. 194:187-197.) To test whether this gene is able to increase infection of LNCaP, we added a peptide from this gene into the infection process of LNCaP. Our results demonstrated that a peptide with 15 amino acid residues (YDSVLALSAALQATR) significantly increased LNCaP infection (Fig. 2). Using similar approaches, we have identified that another peptide with sequence of RGCICRCIGRGICRCIG can also significantly increase LNCaP infection (Fig. 2). We also used these two peptides to check the gene delivery efficiency of human cervical

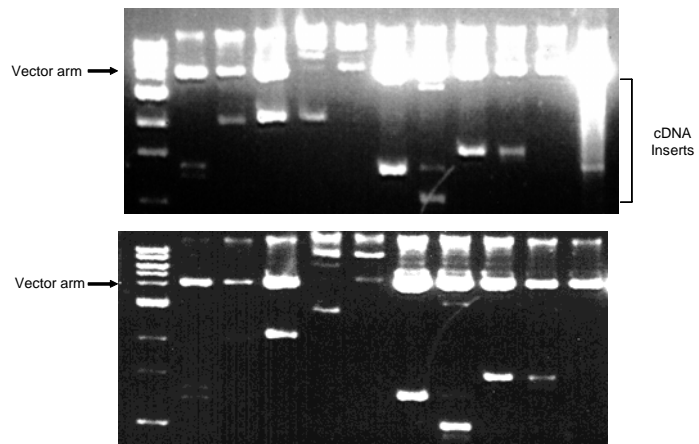


Fig. 1. Checking cDNA inserts of the cDNA library. The eukaryotic cDNA expression cloning kit was purchased from the Invitrogen. The cDNA inserts can be released by digestion of Kpn I restriction endonuclease. As the figure shown, more than 70% clones have cDNA inserts with the sizes of greater than 1 kb.

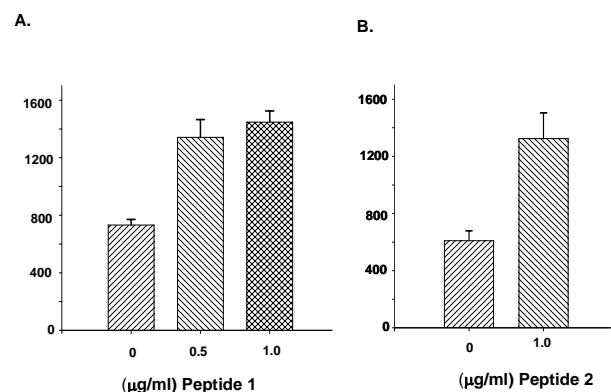


Fig. 2. Two peptides significantly increase LNCaP cell infection. By adding a peptide with sequence of RGCICRCIGRGICRCIG (panel A) or YDSVLALSAALQATR (panel B) into LNCaP cell culture medium during gene delivery by using lentiviral vectors, infection of LNCaP cells can be significantly increased.

peptide with sequence of RGCICRCIGRGICRCIG can also significantly increase LNCaP infection (Fig. 2). We also used these two peptides to check the gene delivery efficiency of human cervical

cancer cell line HeLa, and found that these two peptides could not increase the gene transduction of HeLa cells (Data not shown), suggesting that these peptides stimulate gene delivery in a tissue-specific manner.

In the third year, we worked on using gp41 HIV glycoprotein to fuse with these two peptides in a lentiviral vector. The gp41 protein can present the identified peptide on the surface of the lentiviral vector as shown in Fig. 3. We found that by fusing our peptides with HIV gp41 protein, we could increase gene delivery by 30% to 70% into LNCaP prostate cancer cells (Fig. 3C).

Although the the fusion of the peptides increase gene delivery efficiency by 30% to 70% , it may still meet our requirement to tissue specific-delivering genes into prostate cancer cells. We tried to use a new strategy to identify genes in HOT cDNA library. Our original hypothesis was that only one protein is required for tissue-specific delivery of lentiviral vectors into LNCaP prostate cells. Based on the binding and entry methods of HIV, influenza virus and Sindbis virus, the likely mechanism for a binding-specific viral entry includes two steps: 1) viral binding to its receptors on the surface of the target cells, and 2) viral entry. It is very likely that the protein that is responsible for HOT-derived virus to infect LNCaP cells contains two subunits. One is responsible for vectors to bind to LNCaP prostate cancer cells, and the other one is responsible for viral envelope to fuse into the target cells.

Based on such assumption, we designed new approach. We used influenza HA protein as the fusion protein. The approach is diagrammed in Fig. 4. We use influenza HA protein instead of Sindbis E1

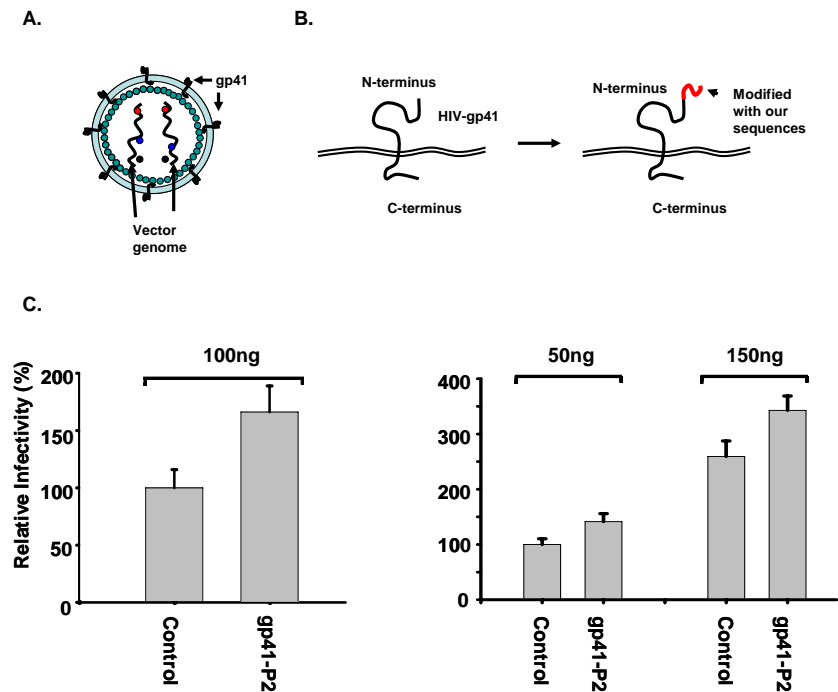


Fig. 3. Strategy of our approach to use two peptides with sequence of RGCICRCIGRGCICRCIG or YDSVLALSAALQATR to modify lentiviral envelope protein gp41. A) A diagram of a lentiviral vector. B) Attachment of our peptide to gp41 at the N-terminus. C) Increased infectivity by peptide YDSVLALSAALQATR. LNCaP cells were infected by a EGFP-expression lentiviral vector with gp41-P2 envelope protein on viral surface or with no gp41-P2 (control) on viral surface. The left panel, Cells in 24-well plates were infected by viral vector of 100 ng p24 reading. The right panel, LNCaP cells were infected by 50 and 150 ng (p24 count) of lentiviral vector from 293T.

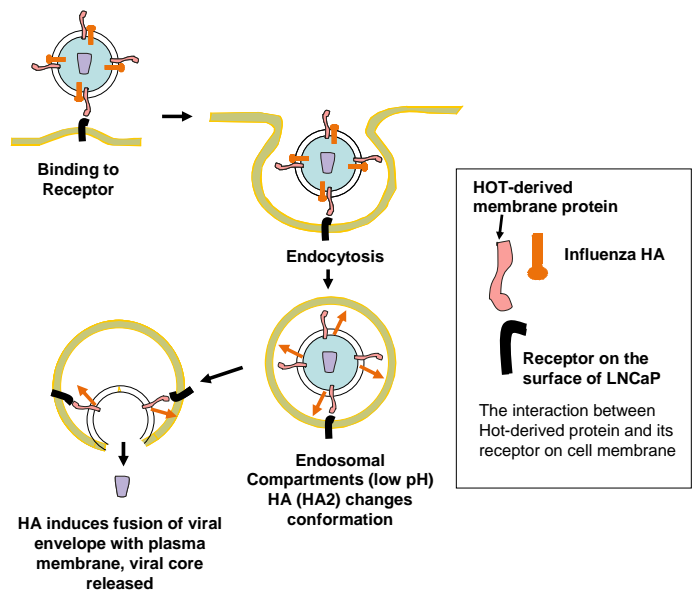


Fig. 4. A diagram to combine Sindbis E1 protein with HOT cDNA library to generate prostate cell-specific vector.

(another widely used fusogen protein) because our results demonstrated that the integration of Sindbis virus envelope protein into lentiviral vector greatly increased the infection background. Our results demonstrated that the HA protein has much lower background. We expected with this system, we should be able to find the protein that can specifically bind to prostate cells. If our cDNA library contains such a protein, we should be able to identify it out. When this protein is work together with influenza HA protein, the lentiviral vector will significantly increase its infectivity in transducing prostate cells.

During the final year, we have screened 2448 HOT cDNA colonies. We have not identified the colonies that showed significant effect in gene delivery. It is difficult to predict that whether by screening more cDNA colonies we can obtain the gene. Because the funding is used up, we were not able to continue the screening.

### **Key Research accomplishments:**

As described above, we have tried all the best to complete the tasks. We have followed the plan to make cDNA library and to screen the library. However, we did not identify the gene we want. As we predicted in our research plan in our grant application, we also expect the risks of our project. To screen a gene is never easy.

Although the main goals of this project are not achieved, the researches have provided valuable information in prostate cancer and HIV researches. One paper related to using peptides to increase delivery efficacy by lentiviral vectors has been published in *AIDS Research and Human Retroviruses*. Another manuscript regarding a protein from LNCaP related to lentiviral infection of LNCaP cells were in press in "Urology". Due to NIH extremely tight of fundings, although some results have been used to apply research grants, none of them have been funded. The two papers related to this project have been published or going to be published in the following journals:

1. Wang Q, Wang W, Zheng J, Tabibian S, Xie Y, Song J, Waring AJ, Chiu R, Yang OO, Chen ISY, Lehrer RI, and Pang S. Paradoxical Effects of Two Theta-Defensins on HIV Type 1 Infection. *AIDS Research and Human Retroviruses*, 2007 23:508-14.
2. Wang, Q, Zheng JY, Kreth J, Kamata M, Campbell RA, Xie Y, Chiu R Berenson JR, Shi W, Chen ISY, and **Pang S**. Regulation of PSA expression by a claudin-7-binding protein, JAM-A. *Urology*, 2008 *in press*

### **Reportable Outcomes:**

As described, two papers related to this project have been published or going to be published.

### **Conclusion:**

Although lentiviral vectors prepared from HOT cell line demonstrated much higher efficiency in gene delivery into prostate cancer cells, the mechanisms may be involved in two or even more membrane proteins on the surface of HOT cells. Therefore, use a simple gene screening strategy to identify these proteins is not a correct approach. However, the membrane protein claudin-7 on the surface of LNCaP can be used in gene delivery. By characterizing claudin-7 in detail, we find that another gene, JAM-A is associated with claudin-7. Because both JAM-A and claudin-7 regulate PSA expression, by inhibiting these two genes, we may be able to inhibit prostate cancer progression. Therefore, the studies provide information to design new strategies to treat prostate cancer.